This Month in Genetics

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TGF-β Signaling in the Balance

The discovery of excessive TGF-β signaling in Marfan syndrome has been an unexpected turn of events in our understanding of this disorder and has led to clinical trials that aim to turn down this pathway with the hope of influencing disease progression. Paradoxically, however, inactivating mutations in *TGFBR1* and *TGFBR2*, the genes encoding the TGF-β receptors, cause Loeys-Dietz syndrome, which shares many features with Marfan syndrome. This begs the question: how could turning up and turning down the same signaling pathway result in similar outcomes? The recent discovery of a novel and related disorder suggests a resolution to this paradox. Groups led by Dianna Milewicz and by Bart Loeys and Hal Dietz have uncovered mutations in *TGFB2* as a cause of thoracic aortic aneurysms. Although many of the mutations, including some full gene deletions, are haploinsufficient, they are associated with increased TGF-β2 expression and signaling. Further, Lindsay et al. found that *Tgfb2* haploinsufficiency exacerbated aortic dilatation in a mouse model of Marfan syndrome, arguing that the two mutations contribute to the same pathogenic mechanism. The likely explanation for this seeming paradox is a model in which reduced function of a component of the TGF-β-signaling pathway leads to an excessive compensatory TGF-β-signaling increase that is at the heart of Marfan, Loeys-Dietz, and related syndromes.

Boileau et al. (2012). Nat Genet. 44, 916–921.
Lindsay et al. (2012). Nat Genet. 44, 922–927.

Separating True Mutations from Sequence Artifacts

Next-generation sequencing has a substantially higher error rate than does traditional Sanger sequencing, and this fact must be managed in the analysis of genomes. Particularly when one is looking for a rare mutation in a DNA mixture, sequence changes that are detected by next-generation sequencing are much more likely to be a result of technical artifacts than of true mutations in the DNA sample. Schmitt et al. figured that they could circumvent this problem by using a naturally occurring check of the DNA sequence: the complementary DNA strand. True mutations, they reasoned, would be present as complementary changes in the two strands of DNA. Although other technologies have assessed sequence in the forward and reverse directions, Schmitt et al. came up with a way to tag the paired strands before any other manipulations, meaning that they can control for errors that occur from the first round of amplification and beyond. To do this, they ligated sheared, double-stranded DNA with distinct randomized tag sequences on each end. The DNA can then be amplified and sequenced, and the tag sets can be used for identifying the related DNA strands. In experiments using M13 DNA, this approach (compared to more standard approaches to sequence analysis) has drastically reduced the estimated mutation frequency and brings this frequency in line with what has been established experimentally for M13. In a mixing experiment, duplex sequencing can accurately detect one mutant sequence per 10,000 wild-type molecules. In a human genetics context, this type of approach could be useful for assessment of DNA mixtures including mitochondrial DNA—something that is explored by the authors—or somatic mosaicism.

Schmitt et al. (2012). Proc Natl Acad Sci USA. Published online August 1, 2012. http://dx.doi.org/10.1073/pnas.1208715109.

Play On

Because of the risk of cardiac arrhythmia and sudden death, individuals with long QT syndrome might be advised to discontinue participation in sports. In the United States, this recommendation is based on the patient's long QT interval, as well as whether the patient has an implantable cardioverter defibrillator or symptoms due to a long QT. European guidelines are even more strict and are solely based on a long QT interval. Johnson and Ackerman wondered what the outcomes were in patients who chose to voluntarily ignore these recommendations and continue sports participation. They had outcome information on a total of 130 competitive athletes with long QT syndrome; 70 were competing within the United States guidelines but contrary to European guidelines, and 60 were participating contrary to both guidelines. Over a mean follow-up period of 5.1 years, only one sports-related event was recorded, and this was in a boy with an extreme QT interval and a history of cardiac arrest. Overall, this is equal to one sports-related event in 331 athlete years, which at least provides families with an estimate of sports-related risks.

Johnson and Ackerman. (2012). JAMA. Published online July 21, 2012. http://dx.doi.org/10.1001/jama.2012.9334.

Who, Which, Why?

Although the key gene that is mutated in classic familial adenomatous polyposis is *APC*, not all individuals with *APC* mutations have classic polyposis with more than

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100 adenomas. Overlapping in the phenotypic spectrum of *APC* mutations are cases of colorectal adenomas due to recessive mutations in *MUTYH*, and some people with multiple adenomas have mutations in neither gene. In a group of individuals with these adenomas, then, how can one tell which gene to analyze and in whom? To tackle this issue, Grover et al. related the mutation frequencies for both genes to phenotype in a sample of more than 8,000 individuals with multiple colorectal adenomas. Although as might be expected, *APC* mutations were increasingly common as the number of adenomas increased, and as the age of diagnosis of adenomas decreased, the prevalence of *MUTYH* mutations was lower and fairly constant relative to adenoma count. The authors developed a multinomial logistic regression model that can be used for assessing the probability of finding a mutation in either gene on the basis of an individual's phenotype and family history. Unfortunately, even if everybody with colorectal adenomas received genetic testing for *APC* and *MUTYH*, a significant fraction of inherited risk for this condition would still not be detected. In this cohort, 18% of individuals with 1,000 or more adenomas—by definition, classic polyposis—had a mutation in neither *APC* nor *MUTYH*. When it comes to adenomas, this analysis does provide some guidance as to who should be sequenced and for which genes, but this is far from cut and dried.

Grover et al. (2012). JAMA 308, 485–492.

Ciliopathy Due to Defect in DNA-Damage Response

Although the primary cilium is not a cellular organelle that most of us learned much about in biology class, increasing amounts of data over the last few years have given cilia much more prominence. Mutations in many different components of this structure, as well as in those that assemble the structure, lead to a class of disorders known as ciliopathies, which tend to manifest as groups of symptoms that can include retinal dysfunction, renal problems, brain malformations, and others. One of the ciliopathy phenotypes is nephronopthisis, a kidney disease that can be attributed to mutations in several genes related to cilia. Even so, a significant fraction of nephronopthisis cases cannot be explained by known ciliary components, leading Chaki et al. to use a combination of homozygosity mapping and exome sequencing to find novel causes of this phenotype. Lo and behold, this approach has led them to link this ciliopathy to a new function: DNA-damage repair. They found nephronopthisis-associated mutations in three separate genes that all function in the DNA-damage-response pathway and confirmed their association with the ciliopathy phenotypes via knockdown experiments in zebrafish. I, for one, am starting to think of cilia as cellular tentacles that reach into many different and interesting aspects of biology.

Chaki et al. (2012). Cell 150, 533–548.

This Month in Our Sister Journal

A Triplet Expansion You Might Actually Want to Have

From autism to congenital heart disease, copy-number variation at the cytogenetically complex segment of chromosomal region 1q21 has been implicated in a range of phenotypes. Within this genomic segment is a repeated element encoding the DUF1220 protein domain, whose copy number has expanded specifically in the human lineage. In recent work published in *G3*, Jim Sikela's group described the evolutionary history of DUF1220 domains. They define six clades of DF1220 sequences and report that the hyperamplification of this domain is restricted to a specific tandemly repeated triplet of DUF1220 sequences. Although this triplet arrangement is present in the chimp and gorilla genomes, its expansion is unique to humans; this might be due to the fact that this chromosomal region contains other human-specific features, including a pericentric inversion from 1p11.2-q21.2 and a polymorphic expansion of heterochromatin. Beyond its human-specific evolutionary structure, though, does the expansion of this domain have anything to do with what makes humans unique? In this issue of *AJHG*, another paper by Jim Sikela's group suggests that maybe it does. They used high-density comparative genomic hybridization arrays to zero in on the individual components of chromosomal region 1q21. This analysis implicates DUF1220 copy number in determining human brain size and suggests that reductions in DUF1220 copy number are at the heart of microcephaly due to deletions at chromosomal region 1q21. Conversely, increases in DUF1220 copy number are correlated with macrocephaly due to duplications in the same region. Thus, as this genomic segment expanded, so might have the human brain.

O'Bleness et al. (2012). G3 (Bethesda) 2, 977–986.

Dumas et al. (2012). Am J Hum Genet. Published online August 16, 2012. http://dx.doi.org/10.1016/j.ajhg.2012.07.016.